Arthur C. Corcoran Memorial Lecture

Influence of Nitric Oxide and Angiotensin II on Renal Involvement in Hypertension

Edward D. Frohlich

Abstract
Remarkable advances have been made with prolonged antihypertensive therapy in reversing cardiovascular morbidity and mortality. Deaths from stroke have been reduced by 70% and from coronary heart disease by 35%. In contrast, end-stage renal disease resulting from hypertension continues to increase. The explanations for this seeming paradox remain unresolved even though experimental models have demonstrated that certain antihypertensive agents may have beneficial renal and intrarenal hemodynamic effects, but reversal of the intrarenal pathological lesions have not been shown to improve. This discussion summarizes recent studies from our laboratory in aged (73- and 85-week-old) spontaneously hypertensive rats (SHR) with naturally occurring end-stage renal disease and in a model of aged SHR employing nitric oxide inhibition in younger, adult (20-week-old) SHR. Our findings demonstrated that the systemic and whole renal hemodynamics, intrarenal glomerular dynamics, proteinuria, and renal pathological lesions can be prevented or reversed with angiotensin-converting enzyme inhibition therapy but not with hydrochlorothiazide (at similar levels of arterial pressure reduction). The implications and possible mechanisms involved in the development of both naturally occurring and nitric oxide-exacerbated SHR are multifactorial, involving the endothelial nitric oxide system and its interaction with angiotensin II (and possibly bradykinin) among other factors. Moreover, these pathophysiological cellular mechanisms may be shared by the aging process as well as in naturally occurring spontaneous hypertension in the rat and, perhaps, in humans with essential hypertension. Thus, antihypertensive therapy seems to be specific in its ability to prevent and even reverse the pathophysiological derangements of renal involvement in hypertension. Thus, prevention and reversal of end-stage renal disease do not seem to require greater reduction of arterial pressure than with other target-organ involvement. However, they do require specific inhibition of the arteriolar and glomerular lesions produced by the disease.

Key Words
endothelium • glomerular disease • arteriolar disease • L-NAME • nitric oxide • L-arginine • angiotensin-converting enzyme inhibition • nephrosclerosis • hydrochlorothiazide

Renal Involvement in Hypertension

The remarkable effects of antihypertensive drug therapy on the reversal of target-organ involvement from hypertension is a classic success story in hypertension. Outcome analyses have clearly demonstrated the remarkable effects of prolonged antihypertensive therapy on reducing the morbidity and mortality of hypertensive vascular disease from stroke and coronary heart disease.6,7 Not usually commented upon in this context, at least as a follow-up thought, is the additional well-known observation that as the foregoing dramatic reductions in deaths from stroke (by upwards of 70%) and from coronary heart disease (by 35%) have continued, there has been an unrelenting increase in the numbers of patients with hypertension progressing to ESRD.8,9 This is a tremendously disturbing statistic, and it is all the more unsatisfying because it leaves hypertension researchers (fundamental as well as clinical) without a satisfying explanation for this devastating and costly world-wide observation that, at present, defies explanation.10

Nephrologists concerned with this seeming paradox have thus far provided no creditable rationale for the continued rise in patients with ESRD. (Fig 1).8 Although several tentative explanations have been offered among these reasonable hypotheses are target-organ involvement of the kidney by hypertensive vascular disease, may not necessarily be reversible with antihypertensive therapy, goal treatment BPs conventionally chosen for antihypertensive therapy (ie, <140 and 90 mm Hg, systolic and diastolic, respectively) may not be optimal and low enough to affect the kidney beneficially, and the antihypertensive...
therapeutic classes used thus far in the long term and controlled clinical trials that have demonstrated reductions in morbidity and mortality from stroke and coronary heart disease were not specific enough for the kidney. Supporting these contentions are the unwavering long-term epidemiological data of the past 20 years.9 This is not to say that every patient progressing into ESRD does so from long-standing essential hypertension; but the vast majority of patients are hypertensive and are either black or have coexisting diabetes mellitus (Fig 2) 9

Background Experimental Studies

In recent years, a number of experimental models have been developed for producing ESRD. In brief, they have usually involved induction of renal failure with unilateral nephrectomy and extirpation of contralateral renal tissue with or without additional salt loading, steroid administration, renal infarction, or administration of nephrotoxic drugs or chemicals. None of these studies, however, have involved the natural development of renal failure in animals with genetic hypertension. Clearly, and of great clinical relevance, these models cannot be considered to be analogous to the patient with essential hypertension who progresses slowly (or even, on rarer occasions, rapidly) into chronic renal failure. Despite this rather frustrating picture, these studies have led to a very useful hypothesis of the potential dynamics that might be involved with the help of carefully performed renal micropuncture analyses. Thus, these models of hypertension and renal disease have demonstrated that the renal involvement in hypertension is associated with both afferent and efferent glomerular arteriolar constriction, glomerular hypertension and hyperfiltration, leakage of protein molecules across the glomerular capillary wall, progressive glomerulosclerosis, and ultimately renal functional deterioration 11-13

In our earlier micropuncture studies involving the 20- to 23-week-old male SHR, we demonstrated abnormal responsiveness of the SHR afferent and glomerular arterioles to α-adrenergic stimulation and inhibition14,15 as well as to improve renal and glomerular dynamics after acute or prolonged administration of a calcium antagonist or an ACE inhibitor.16,18 However, these relatively younger SHR did not exhibit any evidence of increased efferent arteriolar resistance, elevated glomerular hydrostatic pressure, or proteinuria,16-19 which had been postulated earlier.11-13 Therefore, armed with the knowledge that when the SHR becomes 1 year old, it naturally develops marked proteinuria and impaired renal excretory function that is associated with pathological evidence of glomerular injury,20,21 we embarked on a series of studies that provide the substance of this lecture. But, before describing those studies, a word is necessary concerning the rationale for this protocol. Over the past 15 or so years, we had reported a series of studies on another target-organ involvement from hypertension—the pharmacological reversal of increased left ventricular mass in the SHR.22,23 Each of those studies involved the intervention of a pharmacological agent for a period of 3 weeks in male, adult SHR 20 to 23 weeks old Those studies demonstrated from the outset that certain classes of pharmacological agents for 3 weeks were sufficient to reduce left ventricular mass. Most important, we had selected the 3-week period of treatment because we had postulated earlier that participating in the development and reversal of left ventricular hypertrophy (LVH) were nonhemodynamic and hemodynamic factors.24 If early reversal of LVH were demonstrated, it would be likely to be independent of hemodynamic factors of pressure and afterload reduction.25 Thus, the following studies concerned with renal involvement in hypertension were similarly designed to determine whether antihypertensive therapy for 3 weeks would be effective in reversing the renal pathological lesions as well as the associated hemodynamic and micropuncture glomerular dynamic defects.

The SHR: A Model of Renal Involvement in a Genetic Form of Hypertension

Naturally Occurring ESRD

Having been satisfied that changes associated with naturally occurring ESRD do, in fact, occur in the SHR,20,21 we set aside a number of adult, male, 20-week-old SHR to age for 1 additional year after our usual study age of 20 weeks. This would permit us to determine whether the systemic and renal hemodynamics as well as glomerular dynamic changes could be associated with that period of aging.26 These studies clearly demonstrated that by 73 weeks of age, the SHR (in contrast to its normotensive Wistar-Kyoto control of the same age and sex) developed severe glomerular hypertension and ischemia associated with marked afferent and efferent arteriolar constriction, glomerular sclerosis and arteriolar fibrinoid necrosis, and severe proteinuria. Moreover, an equal number of male littersmates of these aged SHR, when treated with the same ACE inhibitor used earlier in younger SHR,18 significantly reversed these marked systemic and renal hemodynamic and glomerular dynamic alterations, as well as the associated pathological lesions and the severe proteinuria within the 3-week treatment.26

L-NAME Model for the 73-Week-Old SHR

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- **Ang II** = angiotensin II
- **FSRD** = end-stage renal disease
- **L-NAME** = N^ ω-nitro-L-arginine methyl ester
- **NO** = nitric oxide
- **SHR** = spontaneously hypertensive rat(s)

### Selected Abbreviations and Acronyms

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### Observed and Projected Treated Incidence Rates, 1984-1993

![Graph showing observed and projected incidence rates of ESRD in the United States](http://hyper.ahajournals.org/)

- **Patients per Million Pop./Year**
- **Calculated**, **Observed**, **Projected**
- **A Trend = 8.69% per year**

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*Fitted to an exponential curve*
determined whether the changes affected by the additional year of aging could be accelerated by 3 weeks of NO synthase enzymatic inhibition.27 NO had been shown earlier to be the major source of the endothelium-derived relaxing factor that plays a critical role in local circulatory control, even in the kidney.28-31 Moreover, other studies had shown that responses to endothelium-dependent vasodilators were impaired in the SHR, possibly the result of reduced endothelium-derived NO or increased endothelium-derived constricting factor.32-35 We therefore determined whether inhibition of the synthesis of endothelium-derived NO in the 20- to 23-week-old SHR might mimic the findings that we had demonstrated in the aged SHR with naturally developing ESRD.36 To test this hypothesis, we inhibited the synthesis of endothelium-derived NO with L-NAME for 3 weeks (50 mg/L drinking water prepared freshly each day). These studies demonstrated that the L-NAME produced marked proteinuria associated pathologically with severe hypertensive nephrosclerosis that was manifested physiologically by intense afferent glomerular arteriolar constriction with severe afferent arteriolar fibrinoid necrosis, segmental glomerular hyalinosis and sclerosis, and renal ischemia.27 Significant efferent glomerular arteriolar constriction was also produced but occurred without development of glomerular hypertension because of the more intense afferent arteriolar constriction that was associated with a significantly reduced renal blood flow and single nephron plasma flow. Thus, the 3-week intervention period with L-NAME mimicked the renal and intrarenal hemodynamic and glomerular dynamic alterations as well as the pathologic and proteinuric findings we had observed earlier in the 73-week-old SHR (Figs 3 through 6).26,27

Pharmacological Interventions

Next, it was of interest to know whether treatment with the same ACE inhibitor that we had used in the earlier two studies18,26 would reverse the renal alterations that were associated earlier with L-NAME. To accomplish this, the same ACE inhibitor was administered either for 3 weeks contemporaneously with the L-NAME (a prevention intervention) or after the 3-week L-NAME treatment (a reversal intervention). These studies demonstrated that the cotreatment as well as the posttreatment with that ACE inhibitor did in fact produce equivalent reductions in arterial pressure and total peripheral resistance that were associated with significant decreases in both afferent and efferent arteriolar resistances, nephrosclerosis pathological scores (of both the glomeruli and the arterioles), and 24-hour urinary protein excretion.36 Most notably, ACE inhibition cotreatmentwith L-NAME completely prevented the renal glomerular hemodynamic alterations associated with L-NAME, and when it was given immediately after the L-NAME, it also reversed the glomerular injury scores that were observed in the same SHR earlier by renal biopsy following L-NAME. These changes were also associated with reduced smooth muscle α-actin deposition (by immunochemistry). Thus, these data demonstrated that ACE inhibition not only prevented but also reversed the L-NAME–exacerbated severe nephrosclerosis in the SHR, as indicated by improved systemic and nephrological findings.
renal hemodynamic, glomerular dynamic, proteinuric, and pathological alterations. In contrast to the foregoing findings with the ACE inhibitor were our very recent observations with the daily administration (by gastric gavage) of hydrochlorothiazide administered for 3 weeks following the 3-week L-NAME period of treatment. In these studies, the diuretic achieved a reduction in arterial pressure similar to that produced by the ACE inhibitor; however, renal microscopy studies revealed an increased glomerular capillary pressure that was associated with a further increase in efferent (but unchanged afferent) glomerular arteriolar resistance as compared with the L-NAME control SHR group. Furthermore, the thiazide diuretic significantly increased the glomerular injury score further (without affecting the arteriolar injury score) that was also associated with a further increase in urinary protein excretion. The pathological studies showed further that the increased glomerular hydrostatic pressure was associated with more severe glomerulosclerosis associated with increased fibronectin and smooth muscle α-actin deposition. Thus, our pharmacological studies clearly demonstrated that whereas an ACE inhibitor could prevent and reverse the systemic and renal hemodynamic and glomerular dynamic alterations as well as the pathological renal lesions associated with naturally occurring and L-NAME–provoked ESRD in the SHR, the thiazide diuretic exacerbated the glomerular lesions as well as the hemodynamic, functional, and pathological indexes of the disease (Figs 3 through 6).

To provide further insight into the underlying mechanisms that could be involved with these changes, we administered L-arginine (2 g/L drinking water) for 3 weeks to 85-week-old male SHR (not given L-NAME). Although these data have not yet been published, they have been presented at this meeting of the Council. The data demonstrated that the 3-week course of L-arginine treatment markedly reversed the severe naturally occurring nephrosclerosis in these rats as well as the cardiac and renal hemodynamic alterations. Although the mean arterial pressure did not remain reduced to levels that were observed earlier with an intravenous infusion of L-arginine (300 mg/kg body wt over 30 minutes) during the baseline, control study period, the acute intravenous response was restored after the 3-week oral treatment by a repeat intravenous infusion of L-arginine. Moreover, the 3-week treatment period significantly improved the glomerular arteriolar injury score (but not arteriolar injury) while reducing the proteinuria.

Interpretation of the SHR Findings

The results of these studies strongly support the following three concepts. First, reversibility of ESRD secondary to naturally developing hypertension (or, for that matter, in L-NAME–exacerbated hypertension) in the SHR is possible with antihypertensive therapy. Second, the reversibility of systemic and renal hemodynamic as well as glomerular dynamic, pathological, and proteinuric alterations were not necessarily dependent on dramatic reductions in systolic or diastolic pressure any more than with other target-organ involvement. And third, the demonstrated pathophysiological reversibility seems to be more dependent on the mechanism of action of the antihypertensive drugs or drugs employed and can be achieved in as short a time period as 3 weeks in the SHR. Indeed, this relatively short-term reversibility had been shown earlier with the reduction of left ventricular mass (in the SHR), which pointed to the locally mediated nonhemodynamic mechanisms of the antihypertensive drugs. Thus, whether in the naturally occurring hypertension of the aged SHR or in the exacerbated hypertensive disease produced by NO inhibition in the endothelium of younger, but adult, SHR, impairment of the generation of Ang II (eg, with an ACE inhibitor) seemed to favor that reversibility. In contrast, the thiazide diuretic, which enhances generation of Ang II through its stimulation of the renin-angiotensin system, exacerbated the pathophysiological alterations of the renal disease. Since a local renin-angiotensin system exists within the kidney, our findings in the SHR seem to favor the concept that progression of the

**Fig 5.** Intrarenal hemodynamic responses of 23-week-old male SHR that were untreated (open bars), given L-NAME (50 mg/L) in drinking water (open crosshatched bars), given L-NAME with an ACE inhibitor (ACEI) (stippled bars), or given L-NAME with hydrochlorothiazide (HCTZ) by gavage (closed crosshatched bars) for 3 weeks. All bars represent mean±1 SEM. PG indicates glomerular pressure; SFP, stop-flow pressure; RA, afferent glomerular arteriolar resistance; and RE, efferent glomerular arteriolar pressure. **P<.01 vs control; TP<.01 vs L-NAME; xP<.05, xxP<.01 vs ACEI.

**Fig 6.** Urinary protein excretion (U_prox) in 24 hours and pathological changes in 23-week-old male SHR that were untreated (open bars), given L-NAME (50 mg/L) in drinking water (open crosshatched bars), given L-NAME with an ACE inhibitor (ACEI) (stippled bars), or given L-NAME with hydrochlorothiazide (HCTZ) by gavage (closed crosshatched bars) for 3 weeks. All bars represent mean±1 SEM. GIS+AIS indicates glomerular injury score plus arteriolar injury score. **P<.01 vs control; xP<.05, xP<.01 vs L-NAME; xxP<.01 vs ACEI.
hypertensive glomerular disease occurs in association with
generation of Ang II despite a reduction of systemic arterial
pressure similar to that which was also achieved by
the ACE inhibitor.  

Our experimental data at the least provide some tenta-
tive mechanistic explanations for the rise of ESRD in
predisposed patients with hypertension who may be par-
ticularly susceptible to diuretic therapy. Nevertheless, it
may not be appropriate at this time to suggest with firm
conviction that Ang II was the sole pathogenetic factor
responsible for the progression of the hypertensive renal
disease since ACE inhibition alone or with NO interac-
tion may also involve other local mechanisms such as
the local generation of kinins or the secondary effects on
other endothelium-derived factors in the kidney and else-
where.  

To this end, our studies involving the inhibi-
tion of endothelially generated NO may also be co-
participants in the pathophysiological responses reported
herein. Sufficient data are now available to support the
thesis seems to be impaired in experimental animals and
other related endothelium-generated mechanisms.

That aging, per se, of the SHR may be analogous to
suppression of the endothelium-derived generation of NO
is supported by several lines of evidence. Thus, NO syn-
thesis seems to be impaired in experimental animals and
humans with aging as well as secondarily in endothe-
rium-related diseases such as hypercholesterolemia and
atherosclerosis and essential hypertension. The present
and high levels) studies in the SHR with natu-
rally developing hypertensive ESRD (or with L-NAMEx
ercedated ESRD in the younger SHR) provide strong
support for these potential mechanisms, and ongoing
studies are being directed toward these concepts in our
laboratory.

It is appropriate in this lecture that honors Arthur Cor-
coran and the investigative team that offered the multi-
tactorial mechanistic explanation of hypertension (the “mo-
saic” of hypertension) to suggest a similar mechanism
for the underlying factors associated with ESRD (Fig 7).
As with the causation of hypertension, this mosaic is not
exhaustive but involves the interaction of other patho-
physiological processes including those of the aging pro-
cess, atherosclerosis, and diabetes mellitus. It also involves
those specific factors related to race, growth, immune
responses, and lipid metabolism as well as with the gener-
ation of NO, Ang II, free radicals, and other humoral and
therapeutic agents.

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less physiological and pathological studies, this sequence
of studies would not have been possible over the past
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